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1437-Pos Board B167
The Influence of the Form of tRNA on Complex Formation with Porphyrins
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In the current work we have investigated the interaction of meso-tetra-(4N-oxyethylpyridyl) porphyrin (TOEPyP4) and its Zn(II), Cu(II), -metallocomplexes with tRNA which has 2 forms: hairpin structure and spatial reversed "L" structure. The interaction of tRNA from E.Coli with porphyrins is studied by UV/Vis Spectrophotometry and Circular Dichroism methods.
The measurements were performed in 0.1 BPSE and 1BPSE buffers (1 BPSE = 6 mM Na2HPO4^{+} 2 mM NaH2PO4 +185 mM NaCl + 1 mM Na2EDTA), correspondingly μ = 0.02M and μ = 0.2M, pH 6.57. (tRNA has hairpin form at μ = 0.02M and reversed "L" structure, when μ = 0.2M).
From the spectrophotometric titration datas the Scatchard binding isotherms for porphyrin-tRNA complexes are built and binding parameters are calculated (N - the number of binding sites per molecule of tRNA, and K - the binding constant). In case of tRNA's hairpin structure for the values of induced CD spectra (at 400-470 nm) for complexes tRNA with TOEPyP4 and CuTOEPyP4 there is an optimum concentration of porphyrins at which the anisotropy of system is maximal. For complexes of ZnTOEPyP4 with tRNA the induced CD spectra are essentially different. The induced CD spectra of complex change a sign and continue to grow (remaining negative) starting from a certain relative concentration. This unusual ICD spectra profile is found for all three porphyrin-tRNA complexes in case of tRNA's reversed "L" structure. It is possible that at high relative concentration of porphyrins the liquid crystal form may exist in the solution.
The binding constants with tRNA in case of hairpin form are an order of magnitude greater than in case of reversed "L" structure. It means that this porphyrin interacts stronger with tRNA when it has hairpin form.

1438-Pos Board B168
Studying Dynamics and Conformational Changes in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy
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Electron Paramagnetic Resonance (EPR) spectroscopy is used to study dynamics and conformational changes in the RNA glycine riboswitch. The dynamic role of the leader-linker interaction within glycine riboswitch conserved sequences is probed through site directed spin labeling and continuous wave EPR. Inter-aptamer and aptamer-expression platform interactions are elucidated through double electron-electron resonance spectroscopy. Incorporation of spin labels is achieved through optimized ligation methodologies allowing synthetically modified RNA to be joined to larger RNA sequences. Expected folding and burial of riboswitch elements will lead to restricted motion of the spin label and, additionally, pulsed EPR experiments yield distance distribution profiles indicating conformational exchange between states in the absence and presence of glycine.

1439-Pos Board B169
Theoretical and Experimental Study of the Conformational Structure of HIV RNA
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HIV-1 genomic RNA has a non-coding 5' region containing sequential conserved structural motifs that controls many parts of the lifecycle. Very limited data exist on their 3-dimensional conformation and then how they work structurally. Recently the novel 3-D structural information of this most highly conserved region of the virus was reported on a promising therapeutic target[1]. In order to learn more on the structure of this RNA, we use single molecule fluorescence, anisotropy imaging microscopy and RNAstructure modelling[2,3] together to monitor its conformational dynamics in physiological condition. We aim to understand with implications for RNA dimerization and protein binding during regulatory steps.
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1440-Pos Board B170
Exploiting Co-Transcriptional Folding and Processing of Nascent Messenger RNA for Modulating Specific Exon Splicing
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RNA splicing, which involves intron removal and exon ligation, is a key step that transforms eukaryotic nascent messenger RNA (pre-mRNA) into mature mRNA. The ability to modulate inclusion or exclusion of specific exons to elicit switching between alternatively spliced variants, restoration or disruption codon-reading frame, and correcting aberrant splicing has applications for both biomedical research and therapy. However, the identification of potent pre-mRNA sites, that when targeted or masked via therapeutics efficiently induce specific exon splicing, remains a challenge. As the transcription elongation of a pre-mRNA, exon recognition and exon splicing occur simultaneously, we hypothesized that the dynamics of co-transcriptional pre-mRNA folding could be a key factor in determining the accessibility of target sites. By analyzing all possible optimal and sub-optimal local secondary structures of the pre-mRNA at each step of transcriptional analysis, we predicted the co-transcriptional accessibility profile and identified possible target sites that have the highest probability of being accessible throughout transcription process. To test the hypothesis, we used glycine decarboxylase (GLDC), an 'oncogenic protein' overexpressed in metastatic lung cancer cells, as a test case. Ten target sites each to induce specific exclusion of one of the two targeted exons of the GLDC gene, were identified to disrupt the codon reading frame to induce nonsense-mediated decay. In our wet experiments on NSCLC (non-small cell lung carcinoma) cell lines, specific exon exclusion were observed when each of the ten sites was masked; of these, three sites induced exon exclusion in more than 80% of total pre-mRNA. Notably, two sites predicted to be ineffective were validated to be so. Our results suggest that co-transcriptional binding accessibility can be used to aid the rational identification of potent target sites for modulating specific exon splicing.

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Structural Polymorphism of (Cag)N Repeat RNA Elucidated using Single Molecule Nanomanipulation
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Abnormal expansions of CAG trinucleotide repeats are responsible for 9 hereditary human disorders including Huntington's disease and a variety of Spinocerebellar ataxias. Disease symptoms typically manifest when the number of repeats exceeds a given threshold (typically 35+ repeats). It has been hypothesized that genetic instability and RNA toxicity arise due to the structurally polymorphic nature of expanded repeats. However, it is technically difficult to study the structure and folding of a heterogeneous population of RNAs. To overcome this problem, we applied a single-molecule approach to monitor folding of individual RNAs with up to 100 CAG repeats using optical tweezers. Compared to RNAs with non-repetitive sequences, a (CAG)n RNA folds slowly and non-cooperatively in multiple back-and-forth small steps, suggesting that the molecule undergoes an extensive conformational search. Most surprisingly, as a (CAG)100 RNA is extended by >100 nm, tension on the molecule remains largely unchanged at ~13.5 ± 0.4 pN, which is not expected for an elastic polymer. This unusual viscoelasticity implies that force is unevenly distributed in the molecular structures of the (CAG)n RNAs which possibly take the form of multi-branched, similarly-sized hairpins. The relatively constant unfolding force indicates that the folding energy landscape is almost flat at the force, and the small force fluctuation suggests that the energetic barriers between different conformations are very low. When a (CAG)n RNA is nanomanipulated to be partially unfolded, the molecule is trapped in a subset of conformations, as evident by the constant value of the mean extension. This observation leads to a hypothesis that the folding of (CAG)n RNAs is dominated by topological constraints, and the existing conformation is affected by preceding structures. Our findings support the structural polymorphism hypothesis for the (CAG)n RNAs and provide evidence for sequential folding of repeated sequences.